

Review Series: New Features of Systemic Vasculitides

# Direct and Indirect Pathogenic Roles of Autoantibodies in Systemic Autoimmune Diseases

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## ABSTRACT

Autoantibody (autoAb) production in patients with systemic autoimmune diseases is a hallmark of disease entity, activity and prognosis. Although a large number of autoAbs have been discovered to date, there is a limited number of autoAbs whose pathogenic roles have been clearly determined. However, intriguing evidence has recently been provided of possible pathogenic roles for anti-neutrophil cytoplasmic Abs (ANCA) against myeloperoxidase (MPO) in ANCA-associated vasculitides (AAV) and for anti-citrullinated protein Abs (ACPAs) in rheumatoid arthritis (RA). Of note, these autoAbs are thought to display both direct and indirect effects on organ failure. Additionally, some autoAbs have been reported to play pathogenic roles in brain damage in patients with neuropsychiatric systemic lupus erythematosus (NPSLE), which is one of the most refractory autoimmune disorders. Thus the binding of autoAbs to a certain sequence of the *N*-methyl-D-aspartate receptor subunit NR2 (anti-NR2 Abs) may directly induce hippocampal neuronal injury. On the other hand, anti-U1 ribonucleoprotein (RNP) Abs might be pathogenic by inducing neurotoxic inflammatory mediators intrathecally. Such autoAb measurements are also clinically meaningful for treatment selection.

## KEY WORDS

anti-neutrophil cytoplasmic antibodies, autoantibodies, RA, SLE, vasculitis

## INTRODUCTION

Autoantibodies (AutoAbs) directed against various human antigens are found in sera from patients with systemic autoimmune diseases. AutoAb production is one of the most important immunological disorders in systemic autoimmune diseases and autoAb measurements also have clinical significance.<sup>1</sup> First, marker Abs that are highly specific for a disease have a diagnostic value. In most of the classification criteria for systemic rheumatic diseases, autoAbs are included as an important item.<sup>2-4</sup> Second, a certain subset of autoAbs is closely associated with severe or life-threatening manifestation of specific disease. In such cases, the presence of autoAbs in serum is informative for determination of treatment. Third, some

autoAbs are correlated to disease activity. Sequential determination of such autoAb titers is helpful for evaluation of treatment efficacy. To date, there have been a huge number of reports suggesting the clinical significance of autoAbs in systemic autoimmune diseases, and techniques for autoAb detection, which have been proved to be clinically meaningful, have been developed. Thus, autoAb tests are indispensable in clinical practice for autoimmune diseases and international recommendations for assessing anti-nuclear antibodies (ANA) have been published.<sup>5</sup>

However, for the majority of autoAbs, it is still unclear whether they are truly involved in the etiopathogenesis of systemic autoimmune diseases or not. Neonatal lupus syndrome may be a good example to cite in this respect, as it is a disease in which it is sug-

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gested that anti-Ro/SS-A Abs can be pathogenic under certain conditions, but the detailed mechanism has not been determined. Drachman suggested a set of 5 criteria that a putative autoAb must fulfil in order to be considered pathogenic<sup>6</sup>: 1) Abs are present in patients with the disease, 2) Abs interact with the target antigen, 3) passive transfer of Ab reproduces features of the disease, 4) immunization with antigens produces a model disease and 5) reduction in Ab levels ameliorates the disease. In other words, both *in vitro* and *in vivo* experiments in addition to clinical evidence are required for confirmation of the pathogenicity of autoAbs.

When organ and tissue damage is induced by IgG-Abs, two different possibilities may be supposed. First, the Abs might bind functional autoantigens (e.g., a receptor) that are expressed on the target tissues and directly suppress these autoantigens or induce an abnormal stimulation (direct pathogenic role). As an example, in patients with Basedow's disease, Abs against thyroid stimulating hormone receptor increase secretion of thyroid hormone. Second, recognition of an autoAb-included immune complex (IC) by FcγR could activate inflammatory mediators (e.g., cytokines) or the complement system, which can induce systemic or local tissue injury (indirect pathogenic role). Under such conditions, the presence of the autoAb inside the target tissue is not always evident.

In this review, we focus on three systemic autoimmune diseases including vasculitis. The autoAbs closely associated with these diseases are discussed from the point of view of direct and indirect pathogenic roles.

## ANCA-ASSOCIATED VASCULITIDES

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) comprise granulomatosis with polyangiitis (GPA, formerly Wegener's granulomatosis), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg-Strauss syndrome). The major target antigens of ANCAs are proteinase 3 (PR3) and myeloperoxidase (MPO). GPA is usually associated with PR3-ANCA, whereas MPA and GPA are associated with MPO-ANCA. Detection of ANCAs in the sera of patients with systemic vasculitis helps to confirm their diagnosis. Whereas ANCAs disappear during inactive disease or remission, an increased titer of ANCA is related to the active disease or to relapse. More than 10 U/mL of PR3-ANCA at 24 months after the initiation of treatment was predictive of relapse within 5 years.<sup>7</sup> Thus, ANCA titers are closely associated with AAV-activity. A report of neonatal vasculitis occurring in a child born from a mother with AAV suggests a pathogenic role for MPO-ANCA.<sup>8</sup> Therefore, transient but severe manifestations specific to vasculitis syndrome in the neonate might be caused

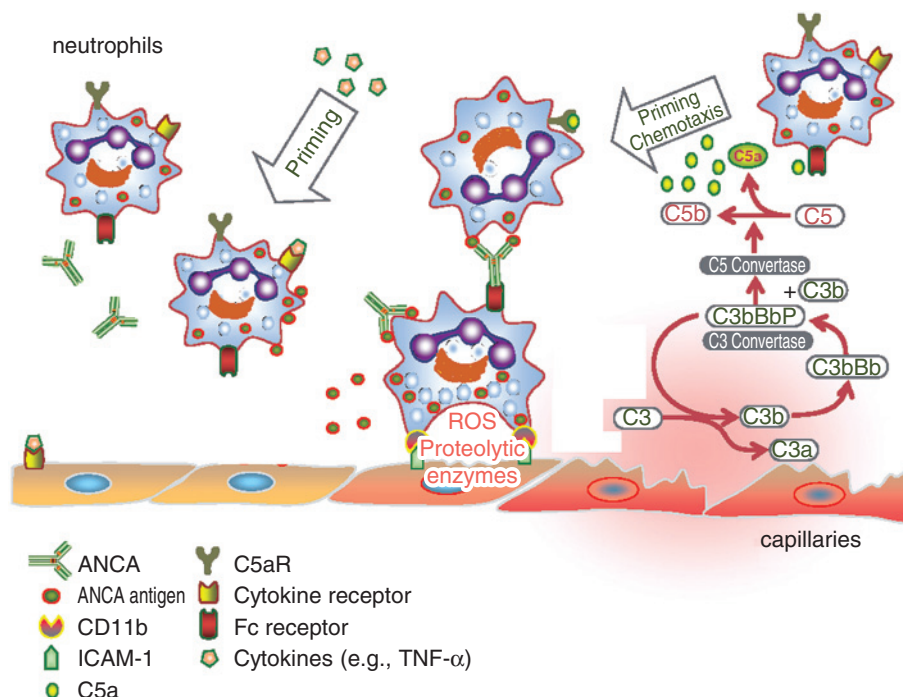
by transplacental transfer of MPO-ANCA. However, a healthy newborn despite transplacental transfer of MPO-ANCA has been reported.<sup>9</sup> The latter observation could suggest that MPO-ANCA is not always pathogenic. Epitope specificity (e.g., aa447-459) recognized by MPO-ANCA may be associated with pathogenesis.<sup>10</sup>

Relapses in AAV occur far more frequently in patients with PR3-ANCA than those with MPO-ANCA irrespective of the associated disease. In addition, PR3-ANCA is associated with granulomatous inflammation and faster decline in kidney function.<sup>11</sup> These clinical differences in relation to the antigenic specificity of ANCAs might be explained by genetic backgrounds.<sup>12</sup>

Whereas the mechanisms of PR3-ANCA-mediated pathogenesis are not well understood, MPO-ANCA is the autoAbs, which appear to fulfill Drachman's criteria and has pathogenicity. The most important characteristics of AAV are pauci-immune necrotizing small-vessel vasculitis and glomerulonephritis, combined with granulomatous inflammation particularly in GPA and EGPA. An animal model of MPO-ANCA-associated AAV, which was made by immunization of MPO-deficient mice with mouse MPO, was developed by Xiao *et al.*<sup>13</sup> These mice developed an immune response to mouse MPO. Subsequently, splenocytes from these mice were transferred into immunodeficient mice. These recipient mice developed pauci-immune necrotizing crescent glomerulonephritis and systemic necrotizing small vessel vasculitis including hemorrhagic pulmonary capillaritis. Neutrophils as well as the presence of MPO were requirements for the induction of lesions in this animal model.<sup>14,15</sup> Thus, MPO-ANCAs have the capacity to further activate primed neutrophils to release proteolytic enzymes (Fig. 1).<sup>11</sup> Priming of neutrophils was induced by low-dose TNF-α, but also can be mediated by other inflammatory mediators (IMs) such as IL-1, IL-18, and the complement degradation product C5a.<sup>16</sup> In order to activate neutrophils, ANCAs not only have to bind to surface-expressed PR3,<sup>17</sup> but also have to interact with receptors for the Fc part of the IgG molecule (FcγR),<sup>18</sup> which are present on the neutrophils. Thus, the pathogenic roles of MPO-ANCA in AAV are mainly explained by indirect rather than by direct effects on small vessel endothelial cells.

A more indirect effect of ANCA has been reported. ANCA can induce the release of microparticles from primed neutrophils followed by binding of these microparticles to endothelial cells.<sup>19</sup> After binding, these endothelial cells show increased expression of adhesion molecules, release of IL-6 and IL-8 and production of reactive oxygen species, which can induce vascular permeability.

Interestingly, it has also been reported that MPO-ANCA directly upregulates adhesion molecule expression in mouse glomerular endothelial cells.<sup>20</sup> The



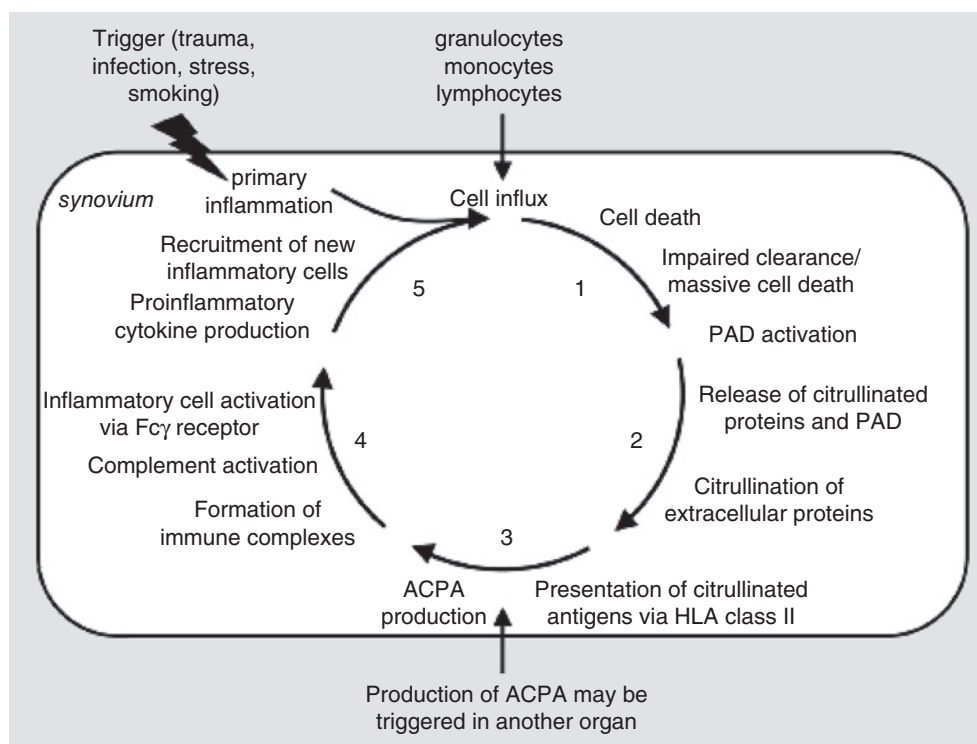
**Fig. 1** Indirect pathogenic effects of anti-neutrophil cytoplasmic antibodies (ANCA) on small vessels. ANCA activates neutrophils by binding to cell surface antigens expressed on cytokine (e.g., TNF- $\alpha$ )-primed neutrophils. As a result, neutrophil degranulation occurs and reactive oxygen species (ROS) are produced. The consequent release of proteolytic enzymes leads to capillaritis. The alternative pathway of complement activation plays a role as an amplification loop of the inflammatory response. ICAM-1, intercellular adhesion molecule 1; C5aR, C5a receptor. Reproduced with permission and modified from: Kallenberg CGM, *et al.* Pathogenesis of ANCA-associated vasculitis: new possibilities for intervention. *Am J Kidney Dis* 2013; 62: 1176-87.

passive transfer of MPO-ANCA to normal mice leads to glomerular damage with neutrophil infiltration.<sup>13</sup> This phenomenon might be the result of cross-reactivity between MPO-ANCA and anti-moesin Abs.<sup>20</sup> By direct binding to moesin, which is a member of the Ezrin/Radixin/Moesin (ERM) family proteins that act as links between the plasma membrane and the actin cytoskeleton, MPO-ANCA leads to an upregulation of endothelial proteins such as intercellular adhesion molecule-1 and E-selectin. Consequently, anti-moesin Abs and/or MPO-ANCA stimulate the production of keratinocyte-derived chemokine and macrophage inflammatory protein-2 by glomerular endothelial cells. These data strongly suggest that MPO-ANCA can directly contribute to the pathogenesis of glomerulonephritis through more than one mechanism.<sup>21</sup>

## RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a systemic autoimmune disease that is characterized by persistent, destructive articular damage and by anti-citrullinated protein antibodies (ACPAs).<sup>22</sup> The 2010 criteria of the Ameri-

can College of Rheumatology/European League Against Rheumatism<sup>2</sup> includes both ACPA and rheumatoid factor (RF) as a single “serology” criterion. In patients with arthralgia, however, the presence of ACPA, but not of RF or of a shared epitope,<sup>23</sup> predicts subsequent arthritis development. When RA patients were divided into 3 groups (ACPA-positive/RF-negative, ACPA-negative/RF-positive, and ACPA-negative/RF-negative) that were compared with normal healthy controls, the level of C-terminal cleavage products for collagen type I (CTXI), which is a marker of bone resorption, was significantly higher in the ACPA-positive/RF negative RA patients than in all other groups.<sup>24</sup> In this study, ACPA-positive/RF-positive RA patients were excluded, because head-to-head comparison of arthritogenic effects between by both autoAbs was intended. Also, ACPA titers were significantly correlated with CTXI levels. Another study examined whether Abs against mutated citrullinated vimentin (MCV) can induce osteoclastogenesis and bone resorption. In *in vitro* experiments, MCV-ACPA induces MCSF/RANKL-mediated osteoclastogenesis. Of note, not only the whole IgG, but also the



**Fig. 2** Indirect pathogenic effects of anti-citrullinated protein antibodies (ACPA) on rheumatoid synovitis (RA cycle). Inflammation of the joint leads to infiltration of immune cells (step 1), which contain peptidylarginine deiminase (PAD) enzymes. PAD activation induces the citrullination of target proteins (step 2). In a small percentage of individuals, citrullinated proteins are exposed to the immune system, which elicits an immune response and anti-citrullinated protein antibodies (ACPA) production (step 3), resulting in immune complex formation (step 4), followed by proinflammatory cytokine activation (step 5). Subsequently, new inflammatory cells are developed and the vicious RA cycle continues. Adapted from: van Venrooij WJ, *et al.* An important step towards completing the rheumatoid arthritis cycle. *Arthritis Res Ther* 2008; 10: 117.

Fab fragment of MCV-ACPA can induce similar bone resorption, suggesting that the possibility that indirect osteoclastogenesis occurs solely through an Fc-mediated effect was excluded. MCV-ACPA was also proven to induce systemic bone loss and osteoclast formation *in vivo*, because *Rag1*<sup>-/-</sup> mice challenged with MCV-ACPA showed a significant reduction in trabecular bone.<sup>24</sup>

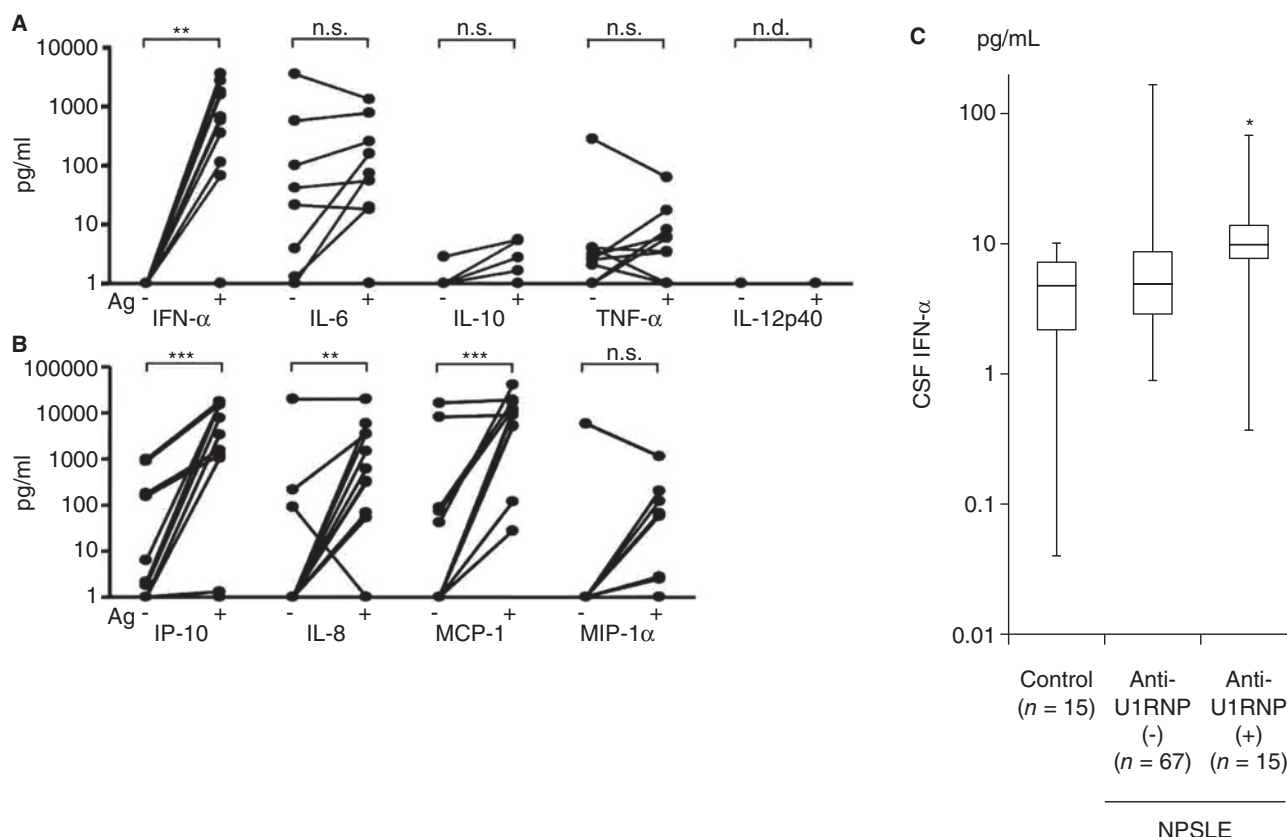
On the other hand, in an *in vitro* model, ACPA-containing ICs induced tumor necrosis factor secretion by human macrophages via engagement of FcγRIIa at the surface of these cells.<sup>25</sup> ACPAs also activate the complement system *in vitro* via classical and alternative pathways. Previous reports clearly showed that citrullination boosts the local inflammatory response at sites of damage or inflammation, indicating indirect pathogenic effects of ACPAs.<sup>26</sup> Unlike RF, ACPA production is mediated by autoreactive T cells.<sup>27,28</sup> Based on these data, van Venrooij *et al.* suggested a vicious cycle in patients with RA ('The RA cycle'), in which ACPA-ICs have a central role in RA etiopathogenesis by continuously promoting the

production of inflammatory mediators (Fig. 2).<sup>29</sup>

Clinical research clearly shows that the presence of ACPA is associated with greater radiological joint damage in patients with RA. Additionally, the Abs against certain subsets of citrullinated proteins (e.g., fibrinogen or vimentin) may meet Drachman's criteria and have arthritogenic effects in patients with RA.

## NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disease, and neuropsychiatric SLE (NPSLE) is a severe life-threatening condition. To date, it has been reported that a certain ANA subset is relevant to central NPSLE. Serum anti-ribosomal P Abs are definitely a useful marker for NPSLE<sup>30</sup> and some reports have shown that anti-ribosomal P Abs in cerebrospinal fluids (CSF) are linked to central NPSLE.<sup>31,32</sup> Regarding the pathogenic roles of anti-ribosomal P Abs, direct effects on Th1 cells<sup>33</sup> and neuroblastoma cells<sup>34</sup> were demonstrated. Ribosomal P protein may be expressed on



**Fig. 3** Anti-nuclear antibodies in CSF may be an inducer of neurotoxic inflammatory mediators. Cerebrospinal fluid (CSF) from neuropsychiatric systemic lupus erythematosus (NPSLE) patients induces neurotoxic inflammatory mediators (IMs). NPSLE CSF was incubated with unprimed PBMCs in the presence (+) or absence (-) of nuclear antigens. Cytokines (A) and chemokines (B) were quantified after 24 hours using a multiplex assay. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . n.s., not significant; Ag, autoantigen; IP-10, Interferon-inducible protein-10; MCP-1, monocyte chemotactic protein-1; MIP-1 $\alpha$ , macrophage inflammatory protein-1 $\alpha$ . (A) (B) Adapted with permission from: Santer DM, *et al.* Potent induction of IFN- $\alpha$  and chemokines by autoantibodies in the cerebrospinal fluid of patients with neuropsychiatric lupus. *J Immunol* 2009; 182: 1192-201 (reference 36). Copyright 2009. The American Association of Immunologists, Inc. (C) We determined anti-U1 ribonucleoprotein (RNP) Abs in the CSF from NPSLE patients. The presence of anti-U1RNP Abs in CSF is associated with an increased concentration of CSF IFN- $\alpha$ , which is neurotoxic. \* $P < 0.05$ .

the neuronal cell surface and anti-ribosomal P Abs can directly contribute to neuronal death.<sup>35</sup> Alternatively, a close correlation of anti-ribosomal P Abs to CSF IMs was strongly suggested in an *in vitro* study.<sup>35,36</sup>

Kowal *et al.* reported that a certain anti-dsDNA Ab subset in CSF, which cross-reacts with the NR2 glutamate receptor, causes apoptotic neuronal death in the mouse hippocampus.<sup>37</sup> In NPSLE, Abs that bind to a specific sequence of the *N*-methyl-D-aspartate receptor subunit NR2 (anti-NR2 Abs) may be the most established neurotoxic autoAbs and may meet Drachman's criteria. Anti-NR2 Abs, which cross-react anti-DNA Abs, can inhibit excitatory synaptic transmissions in the central nervous system.<sup>38</sup> In a mouse model, after a breach in blood brain barrier (BBB) integrity induced by LPS, anti-NR2 Abs that are passively transferred directly attack neurotransmitters.

Also in human lupus, anti-NR2 Abs in CSF, but not in serum, were shown to be involved in diffuse central NPSLE.<sup>39-41</sup> A BBB injury results in an anti-NR2 Ab-mediated severe psychiatric disorder<sup>41</sup> and an attack of serum anti-NR2 Abs on endothelial cells may trigger BBB injury.<sup>42</sup>

On the other hand, our cohort shows that anti-U1 ribonucleoprotein (RNP) Abs are more frequently observed than anti-DNA Abs in the sera of patients with NPSLE. Okada *et al.* previously reported that 13 of 14 patients (7 SLE, 5 mixed connective tissue disease (MCTD) or overlap syndrome, 1 undifferentiated CTD and 1 Sjögren's syndrome) with aseptic meningitis attributed to CTD had serum anti-U1 RNP Abs.<sup>43</sup> Recently, by using an RNA-immunoprecipitation assay (RNA-IPP), we found that anti-U1RNP Abs in CSF could be a useful biomarker of primary NPSLE.<sup>44,45</sup> Compared with ELISA, RNA-IPP is a more sensitive

and specific immunological method for detection of anti-RNA binding protein (RBP) Abs such as anti-U1 RNP, especially Abs against the native form of RBP.<sup>46</sup> Because less than 20 µL of sample is enough for assay, RNA-IPP is also more suitable than ELISA for detection of anti-RBP Abs in CSF, which usually cannot be obtained in large amounts from patients. However, how CSF anti-U1RNP Abs correlate to NPSLE pathogenesis remains unclear. In addition to autoAbs, IMs (cytokines and chemokines) have been found in the CSF of NPSLE patients.<sup>47,48</sup> Previous reports showed that the levels of IFN- $\alpha$ ,<sup>49</sup> IFN- $\gamma$ -inducible protein (IP)-10,<sup>50</sup> IL-8,<sup>51</sup> monocyte chemotactic protein (MCP)-1<sup>52</sup> and fractalkine (CX3CL1)<sup>53</sup> in CSF are significantly higher in NPSLE than in non-NPSLE patients. IFN- $\alpha$  production in SLE is caused, at least partially, by autoAbs binding to RNP particles released from dead or dying cells.<sup>54,55</sup> Interestingly, there was no significant difference in CSF IFN- $\alpha$  levels between serum anti-U1RNP Ab-positive patients and controls. However, the CSF IFN- $\alpha$  level was significantly elevated in patients whose CSF was positive for anti-U1RNP Abs (Fig. 3).<sup>56</sup> Fragosio-Loyo *et al.* reported that CSF IFN- $\alpha$  was not a useful biomarker of central NPSLE.<sup>57</sup> Our data indicated that no elevation of CSF IFN- $\alpha$  levels occurred in patients without CSF anti-U1RNP Abs and that an increased CSF IM levels may depend on the specificity of CSF autoAbs. Although there was no significant association of serum or CSF anti-U1RNP Ab positivity with NPSLE forms in our patients, anti-U1RNP Ab-IC might have an indirect effect on brain tissue as a neurotoxic IM-inducer.<sup>36</sup>

## CONCLUSION

In discussing the pathogenic roles of autoAbs, especially those in systemic autoimmune diseases, indirect effects of autoAbs on the development of disease-specific organ damage should be considered. Even if autoAbs fail to directly react with antigens expressed on the target organ, it is possible that autoAbs that are closely associated with clinical manifestations are involved in the disease etiopathogenesis. For investigation of more effective treatment targets of systemic autoimmune diseases, both future clinical and basic research of autoAbs are warranted.

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